Transcriptional Effectors of the RTK/Ras/MAPK Signaling Pathway in Founder Cell Fate Specification in the *Drosophila* Visceral Mesoderm

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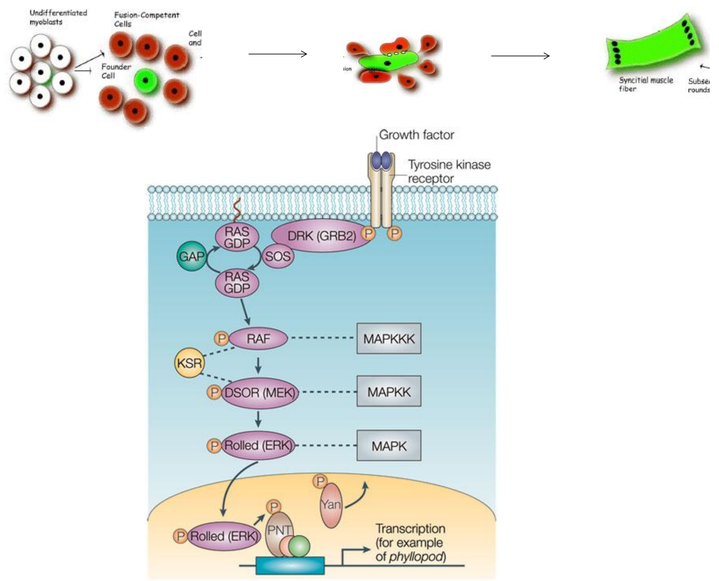
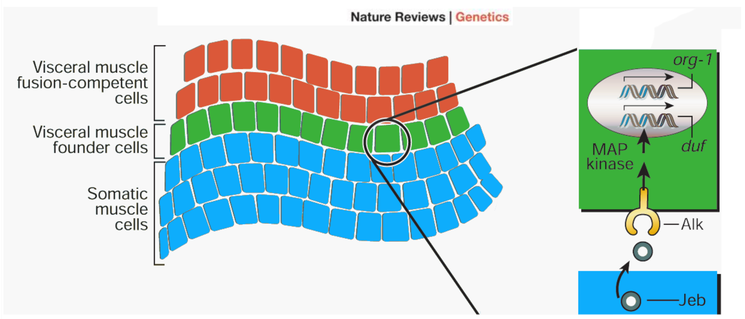
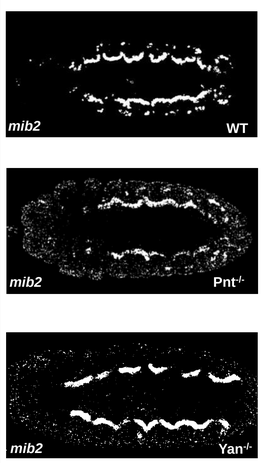
Introduction





In *Drosophila* embryonic muscle development undifferentiated myoblasts differentiate into "founder" cells (FCs) and "fusion competent" cells (FCMs). FCMs fuse into FCs to form a syncytial muscle fiber, with the FCs serving to mandate the identity of the entire fiber [1]. Founder cell specification requires inductive signaling via the Receptor Tyrosine Kinase/Ras/MAP Kinase signaling pathway[2,4].

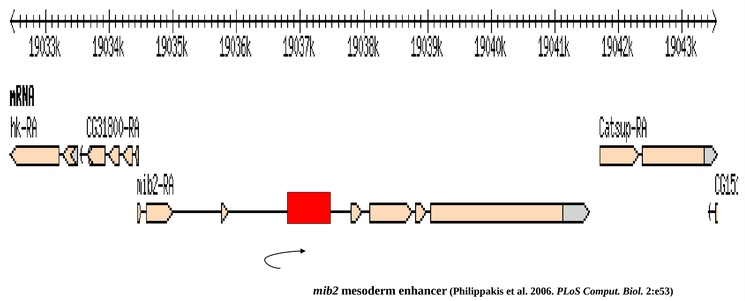
Transcriptional Regulation of the Pathway



In both somatic and heart muscle, the Ets-domain transcription factors Pnt and Yan (Aop) have been shown to be the downstream transcriptional regulators of the RTK/Ras/MAPK signaling pathway. In the visceral mesoderm (VM), however, we have demonstrated that these factors do not play a significant role in mediating the RTK response. To determine the transcriptional effectors of RTK/Ras signaling in visceral FC specification, we are investigating an enhancer region of the FC specific gene, *mib2.*

A *mib2* enhancer

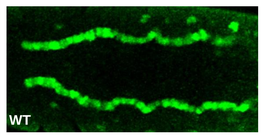
CTATTAAAGCAGCTGCACACGTTTAGGATTAGGTCATGGTATGTGTTCCCCCCTCCCCAA
GTCGCCCTATTCCAGCCTGTGCAGCGAATGCATATGCACATGTCAGGCGCTAGGGCGCGT
GCCATTTTGTCTCCAACCTTTTTTTGAGTGTTTATTTTTAGTTACACGGGTGGGGATCGC
GAGCAGTTACACAGCTCCAGCCGGCCTGATATTTGGGGACGATGTGGAGAGTTTATTTTT
GGACCTTGGTATTCCTCCGACTGGGGTGTGTTGAAAATAGACATAAACAAATTTTCAGAT
GGGAGCCCAGCCATCGGTGCTCCCATTCCTCCCCAGGCCAACATCAAATGTCACCCTCTA
GAGAACGATTCCACATCCACACCCGTGATACGGGGTAATTATAGAGCAGCTGCACGCCCG
AGTCCGAGTGCATAACAAAAATCCGCTTGGAGCCCGAGCCCGAGTCAATGATTATGGGTA
TTTATAGTCACGGCGCGTTGTTTATGAGGGGGAAGTGTGCGCTCTGCATATGTCTGATAT
CCACCGGATGTGGATGTGGGGATGGTGATGCCGATGTACACACAATGCAGCCCTAGGAGC
ATCCTTGTGGCAGCTCTATAGC



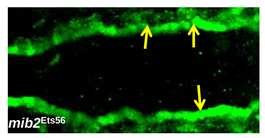
***mib2* mesoderm enhancer (Philippakis et al. 2006. *PLoS Comput. Biol.* 2:e53)**

A *mib2* enhancer was identified based on predicted putative Pnt binding sites [5]. Despite Pnt not playing a role in VM development, We mutated these sites to see if they may play a role in FC development by binding factors other than Pnt.

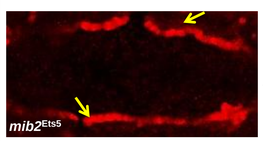
Mutating the *mib2* enhancer



Wild type expression of the FC specific gene *mib2* in the VM.

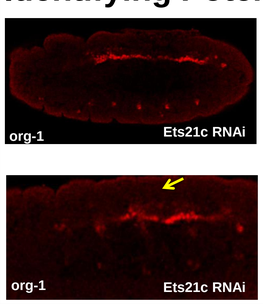


Mutations to sites 5 and 6 together result in expansion of FC population in the VM.



Mutating only site 5 results in the expansion of the FC population in the VM.

Identifying Potential Binders



RNAi was used to knockdown candidates identified by a yeast one-hybrid screen that may potentially bind to site 5. The images show Ets21c knockdown embryos, which results in the expansion of the FC population in the VM, shown by org-1 staining.

Functional Motifs

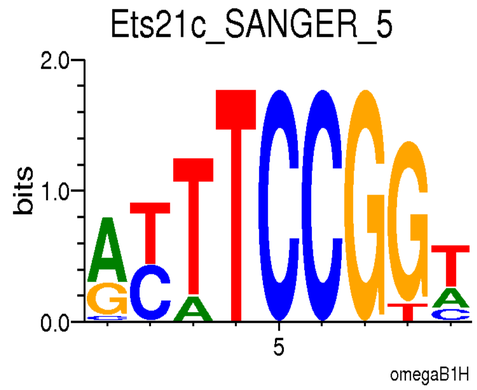
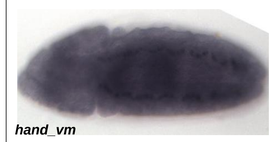
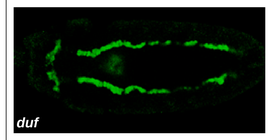
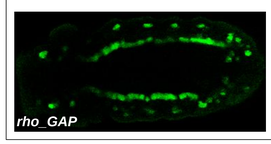


Image of Ets21c binding motif courtesy of FlyFactor Survey. We are currently using the Regulatory Sequence Analysis Tools (RSAT) to search the available FC specific enhancer sequences for Ets21c sites along with other candidate sites.

Other FC Genes and their Enhancers







We along with others have cloned a number of additional enhancers of other FC specific genes so that we can look for commonalities among the various enhancers that might give us clues as to what the important functional motifs are. The images show enhancer driven VM expression in hand_vm [6] dumfounded and rho_GAP embryos.

Conclusions

- FC fate specification in the *Drosophila* VM occurs independently of Pnt.
- Mutations made to a predicted binding site results in the expansion of FC population in the VM.
- RNAi lines have validated potential site 5 binding candidates.
- Bioinformatic approaches are being used to identify possible commonalities and functional motifs among other FC specific enhancers.

References

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[5] Philippakis et al. (2006) PLoS Comput. Biol. 2:e53
[6] Popichenko D, Sellin J, Bartkuhn M, Paululat A. (2007). BMC Developmental Biology. 7:49.

Acknowledgements

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